CERTIFICATE OF COMPETENCE

I, JASON CHRISTOPHER GARRY, a British citizen, identified by Colombian civil registration number 244183 and British passport number **703149572** (expiry date November 12th 2012), certify that my native language is English and that I have been doing translations from Spanish to English for the Universidad Nacional de Colombia's Instituto de Biotecnología since June 1995. Such services have covered the following types of document:

- Translating scientific articles for submission for publication in international journals in English;
- Translating summaries for the *Revista Colombiana de Biotecnología* into English;
- Translating the same type of summaries for the *Revista Colombiana de Fitopatología* into English; and
- Translating summaries and whole articles into English for *Innovar* and the *Cuadernos de Economía*.

I thus certify that I am familiar with both languages (English and Spanish) and that the translation from Spanish to English of the text of the patent entitled, "**Biopolymer based on *Lactococcus lactis* NRRL B-30656: a process for culturing *Lactococcus lactis* NRRL and the biopolymer production process**," is a true and faithful version in English.

The present certificate was issued on the 9th November 2006 at the request of the Instituto de Biotecnología.

JASON CHRISTOPHER GARRY
Civil registration number 244183 issued in Bogota
British passport number **703149572**

CERTIFICACIÓN

Yo, JASON CHRISTOPHER GARRY ciudadano de nacionalidad Británica identificado con la Cédula de Extranjería número 244183 y Número de Pasaporte Británico **703149572** con fecha de expiración Noviembre 12 de 2012, certifico que mi lengua materna es el Inglés y que presto servicios de traducción del idioma Español al idioma Inglés, al Instituto de Biotecnología de la Universidad Nacional de Colombia desde junio de 1995, servicios que contemplan entre otros los siguientes tipos de documentos:

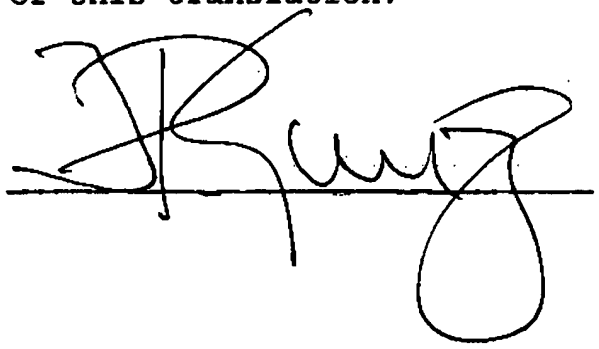
- Traducción de artículos científicos para revistas internacionales publicadas en inglés.
- Traducción al inglés de los resúmenes de artículos de la Revista Colombiana de Biotecnología.
- Traducción al inglés de resúmenes de la Revista Colombiana de Fitopatología.
- Traducción al inglés de resúmenes y artículos completos para la Revista Innovar y para la Revista Cuadernos de Economía.

Por lo tanto atestiguo que estoy familiarizado con los idiomas inglés y español, y que la traducción al inglés del texto de la Patente "Biopolímero con base en *Lactococcus lactis* NRRL B-30656, el Proceso para el Cultivo del *Lactococcus lactis* y el Proceso para la Producción del Biopolímero" es precisa.

La presente certificación se expide a los nueve días del mes de Noviembre del año 2006, a solicitud del Instituto de Biotecnología.

JASON CHRISTOPHER GARRY
Cédula de Extranjería No. 244183

I, the undersigned, Juan Pablo Gonzalez, certify that I am familiar with the Spanish and English languages and that I have made a complete and accurate revision of this translation.



Biopolymer based on *Lactococcus lactis* NRRL B-30656, the process for culturing *Lactococcus lactis* NRRL and the biopolymer production process

Background of the invention

1. Field of the invention

This invention relates to a glucose and fructose polymer and the method for preparing it using a *Lactococcus lactis* strain. The exopolysaccharides are natural glucose and fructose polymers. These polymers can be found in several plants and microorganisms and are useful as emulsifiers, thikener and surfactants in the food and medicaments industries.

2. Description of the state of the art

Fructosans naturally occur in two general forms differentiated by the type of binding between molecules of fructose : inulin, as found in plants, is formed from a column of fructose molecules bound by beta,2-1 links. Levans,formed as microbial products, have a column of fructose molecules bound by beta,2-6 links. The fructosans from plants are smaller (around 100 residues) whilst microbial levans contain more than 3 million residues (Pontis *et al.*, 1985, Biochemistry of Storage Carbohydrates in Green Plants. In: Dey and Dixon (eds). Ch. 5, p. 205. New York, Academic Press).

Microbial Levans are produced with sucrose-based substrates having a variety of microorganisms: *Acetobacters* (Loewenberg, *et al.*, 1957. Can. J. Microbiol., Vol. 3, p. 643), *Achromobacter sp.* (Lindberg, G., 1957. Nature. Vol. 180, p. 1141), *Aerobacter aerogenes* (Srinivasan, *et al.*, 1958. Science. Vol. 127, p. 143), *Phytobacterium vitrosum* (Belval, *et al.*, 1947. 1948. Compt. Rend. Vol. 224, p. 847 and Vol. 226, p. 1859), *Xanthomonas pruni* (Cooper, *et al.*, 1935. Biochem. J. Vol. 29, p. 2267), *Bacillus subtilis* (Dedonder, R., 1966. Meth. Enzymol. Vol. 8, p. 500 and Tanka, *et al.*, 1979. J. Biochem., Vol. 85, p. 287), *Bacillus polymyxa* (Hestrin *et al.*, 1943. Biochem. J., Vol. 3, p. 450), *Aerobacter levanicum* (Hestrin, *et al.*, Ibid.), *Streptococcus sp.* (Corrigen *et al.*, 1979. Infect. Immun., Vol. 26, p. 387), *Pseudomonas sp.* (Fuchs, A., 1956. Nature. Vol. 178, p. 92) and *Corynebacterium laevaniformans* (Dias *et al.*, 1962. Antonie Van Leewenhoeck, Vol. 28, p. 63).

There are some reports of levan being produced at very low levels and having low purity to be used industrially.

Other biological polymers such as xantan and dextran gum have been extensively used in the food industry as stabilisers in emulsions and froth in ice-cream, in salad-dressing, etc. (Sharma, S.C., January 1981. J. Food Tech., p. 59). Extracellular polysaccharides produced by microorganisms offer a variety of uses and potentially low costs.

Small quantities of levan are generally produced by sucrose fermentation using *Actinomyces viscosus* or *Aerobacter levanicum* strains.

Bacillus polymixa generally produces hetero-polysaccharides having different forms of polymers. Genetically modified *E. coli* strains have been used for producing levan (Gay, P. *et al.*, 1983. J. Bacteriol. Vol. 153, p. 1424). Furthermore, other aerobic fermentation methods have also been used for producing levan (Jeanes, *et al.*, U.S. Pat. No. 2,673,828; Gaffor, *et al.*, U.S. Pat. No. 3,879,545; Ayerbe, *et al.*, U.S. Pat. No. 4,399,221). The drawback of such processes is that they produce low product yield and problems related to contamination, thereby industrial processes leading to greater productivity are required.

Description of the invention

The main purpose of this invention was to provide a biopolymer produced by an enzyme transferase having glucose and fructose transfer activity. It was produced from a *Lactococcus lactis* strain (NRRL B-30656) characterised by its high transfer activity, allowing the obtention of the biopolymer by a simple production method which was easy to scale-up. Its method of production consisted of the following steps: **Phase 1**: fermentation with the *Lactococcus lactis* NRRL B-30656 strain in culture medium developed for this micro-organism's growth. **Phase 2**: extracellular enzyme recovery by centrifuging or ultra-filtration. **Phase 3**: biopolymer production by enzyme reaction using sucrose as substrate

and enzyme extract. **Phase 4**: biopolymer purification by precipitation with solvents or ultra-filtration followed by drying the product.

Detailed description of the invention

The object of the invention was to produce a pure biopolymer which was free from polysaccharide contaminants. The biopolymer can be described as a polymer produced by a *Lactococcus lactis* strain isolated from the soil. This strain has high transfer activity, leading to obtaining the biopolymer by a simple process, having greater than 95% purity.

The microorganism. The *Lactococcus lactis* NRRL B-30656 strain was isolated from soil in the present invention by a selection process using a medium containing sucrose as carbon source in which transferase enzyme-producing microorganisms were able to use the substrate and produce polymers, giving a mucoid aspect to the colony. Microorganisms having these characteristics were selected from this medium and purified by isolation techniques involving successive dilutions and plate isolation. The *Lactococcus lactis* NRRL B-30656 strain was obtained from these strains and was used in the present invention.

In accordance with the present invention, the *Lactococcus lactis* NRRL B-30656 strain has been deposited in the *Agricultural Research Service Patent Culture Collection NRRL* Reference Bank; it was assigned registration number NRRL B-30656 by this institution. This strain produces an enzyme having 2-6 U/ml glucose transfer activity, using sucrose as

substrate and also produced a 900-1,100 K Dalton molecular weight glucose and fructose polymer.

The strain was called NRRL B-30656. This strain was isolated and characterised at the Universidad Nacional de Colombia's Instituto de Biotecnología (IBUN). The strain was kept at 4°C in Petri dishes with a culture medium whose composition was: 10-40 g/l sucrose, 7-30 g/l yeast extract, 5-20 g/l 0.05-05 g/l potassium phosphate, 10-100 ppm mineral salts, pH 5-9.

The microorganism was characterised by optical microscopy using Gram staining and electronic transmission microscopy by means of positive staining with uranyl acetate and lead citrate. The biochemical characterisation was done using the computerised MicroScan system, according to that described in Bergey's determinative bacteriology manual (Stanley, W; Sharpe, E; Holt, J. 1994. Bergey's Manual of Systematic Bacteriology, William and Wilkins, Baltimore).

Culture medium. A balance was carried out between carbon source, nitrogen source and certain trace elements for designing and optimising the culture medium for the fermentation with the NRRL B-30656 *Lactococcus lactis* strain. The culture medium provided the microorganism with the nutrients needed for growing and producing the enzyme.

The following concentrations were established, as a result of evaluating culture medium components:

Component	Concentration (g/l)
Salts	
K_2HPO_4	7-30
$FeSO_4.\ 7H_2O$	0.01-1
$MgSO_4.\ 7H_2O$	0.01-0.1
$MnSO_4.\ H_2O$	0.001 – 0.1
$CaCl_2.\ 2\ H_2O$	0.001 – 0.01
NaCl	0.01-0.1
Carbon source	
Sucrose	10-40
Nitrogen source	
Yeast extract	7-30

The pH was adjusted to pH 5-9 using HCl. The medium was sterilised at 121°C for 15 minutes.

Fermentation. The pre-inoculums corresponding to 5-20% inoculum volume were activated from the pure strain kept at -70°C in the medium with 20% glycerol; incubation time did not exceed 10-36 hours, during which time it was necessary to verify the pre-inoculum purity. These cultures were done in stirring flasks, occupying 5-20% of the total volume and incubating them at 20-40°C with 100-400 rpm shaking in orbital shakers. The number of inoculums necessary was determined according to the number and size of the fermenters.

Growth and enzyme production conditions were: 20-40°C temperature and 100– 400 rpm agitation (depending on the fermentation scale).

Aeration. The microorganism that enhances the fermentation is aerobic, meaning that the culture had to be aerated with 0.1 –1 volumes of air per medium volume per minute (vvm) and pH kept between 5 and 9 during fermentation. Culture mediums resulting from this production process had combinations of components for reaching final 10-30 g/l biomass concentration, wet weight, having 2-6 U/ml transfer activity, this being achieved in 6-24 hours.

Enzyme recovery. Extracellular enzyme was recovered by centrifugating at 3,000 -10,000 rpm for 15 minutes or filtration to separate the biomass. Enzyme extract thus presented a 2-6 U/ml transferase activity.

Biopolymer production

Enzyme reaction. Reaction conditions were as follows:

Reaction medium:

50-300 Mm phosphate buffer pH	: 5-9
Substrate	: 5-40% sucrose
Quantity of enzyme	: 10-40% v/v of enzyme extract
Reaction time	: 12-48 hours
stirring	: 100-400 rpm

Biopolymer recovery and purification

The temperature was reduced to 4°C following enzymatic reaction and the biopolymer could be recovered in two ways:

a) Precipitation with solvents

96% ethanol was added to the cold reaction mixture with stirring. The quantity of ethanol added was 1.2- 2.0 volumes of ethanol/ reaction mixture volume.

The precipitated biopolymer was redissolved in half the volume of deionised and distilled water and precipitated again with 1.2-2.0 volumes of ethanol/ reaction mixture volume.

The precipitated biopolymer was redissolved in a third the volume of water and dried by lyophilisation or dried by compressed air at 60°C until reaching 5-6% Humidity.

b) Ultrafiltration

The reaction mixture was submitted to ultra-filtration through a regenerated cellulose membrane having a pore size greater than 10,000 Dalton to eliminate residual glucose and fructose. The biopolymer was then submitted to aspersion drying.

The biopolymer was characterised by high performance liquid chromatography and 10% solution viscosity at 30°C. The biopolymer presented a 7-7.5 minutes retention time using a Shodex SC1011 column at 70°C, 0.6 ml/min flow and HPLC grade water as mobile phase.

The viscosity of a 10% solution at 30°C was found to range from 400-800 centipoises (cP) using a ViscoEasy viscosimeter (Serie L, Schott, Ref. 28.541.120) L2 stem at 50 rpm.

Average DVS (diameter/volume/surface) particle size was 224 micron. The biopolymer had a true density close to that of ssucrose (1.5 mg/ml). It is a material presenting high inter-particle porosity (48%).

Examples

The following examples are given to illustrate the present invention.

Example 1

Isolating and identifying the biopolymer-producing microorganism

A biopolymer-producing bacterium was isolated from soil and identified as being *Lactococcus lactis* NRRL B-30656. 10 g samples were collected from soil and grown in 100 ml liquid medium containing sucrose as carbon source. This was incubated at 30°C with stirring for 24 hours. 4 x 1:10 dilutions were done in saline solution once growth was obtained; the fourth dilution was seeded. This culture was re-seeded in solid medium using the same composition and isolations were done, selecting the colonies showing polymer production. The culture was then transferred to a fresh medium and cultured for 24 hours. The microorganism was kept in a sucrose medium with 20% glycerol at –70°C and by lyophilisation using 10% skimmed-milk, once it had been isolated.

The isolated strain, cultivated in solid sucrose medium, showed the following macroscopic characteristics: clear, cream-coloured, rubbery, circular colonies having a defined edge of around 2 to 3 mm diameter (in 24 hours culture). Gram cocci were observed by microscope via Gram staining; they were occasionally found individually but were generally seen forming groups.

Electronic transmission microscopy characterisation led to observing circular cells in which the cell wall could be differentiated. No special structures were observed (i.e. electro-dense granules, flagella, fimbria, etc).

The strain of the present invention is *Lactococcus lactis* NRRL B-30656, catalogued as GRASS microorganism and shows the following biochemical characteristics:

Test	**Result**
Growth at 10°C	Positive
Growth at 15°C	Positive
Growth at 42°C	Negative
Growth at pH 4.8	Positive
Growth at pH 6.5	Positive
Growth at pH 9.2	Doubtful
Growth in 0.5% NaCl	Positive
Growth in 4% NaCl	Positive
Growth in 5% NaCl	Positive

Growth NaCl 6.5%	Positive
Growth in 10% NaCl	Negative
Growth in 15% NaCl	Negative
Catalase	Negative
Haemolysis	Gamma
Motility	Negative
Vogees-Proskauer	Positive
Aerobic glucose Anaerobic glucose Gas production	Positive Positive Negative
Aerobic lactose Anaerobic lactose Gas production	Positive Positive Negative
Aerobic fructose Anaerobic fructose Gas production	Positive Positive Negative
Aerobic maltose Anaerobic maltose Gas production	Positive Positive Negative

Aerobic manitol Anaerobic manitol Gas production	Doubtful Doubtful Negative
Aerobic galactose Anaerobic galactose Gas production	Positive Positive Negative
Aerobic sucrose Anaerobic sucrose Gas production	Positive Positive Negative
Aerobic xylose Anaerobic xylose Gas production	Doubtful Doubtful Negative
Aerobic rafinose Anaerobic rafinose Gas production	Positive Positive Negative
Ribose	Positive
Trealose	Positive
Sorbitol	Positive
Mannose	Positive

Arabinose	Positive
Arginin	Positive

Example 2

Extract production or enzymatic preparation

1. Fermentation:

a) Microorganism activation

The *Lactoccoccus lactis* NRRL B-30656 microorganism was used for obtaining the transferase enzyme. Bacteria were stored in a cryoprotection solution (glycerol) at -70°C. The strain was slowly unfrozen at room temperature and activated in 50 ml sucrose medium at 30°C for 12 hours and stirring at 180 rpm. 5 ml of this culture were used for two types of seeding. One was seeded in sucrose agar, incubated at 30°C for 24 hours, mucoid characteristics were observed and stored at 4°C; the second was seeded in 100 ml sucrose broth and incubated at 30°C for 12 hours. The latter was distributed in 1 ml centrifuge tubes with 20% v/v glycerol and stored at –70°C, kept for later fermentations. The remaining 45 ml of initial culture were kept in 5 ml vials, by lyophilisation, using sterile skimmed milk as support at 10% concentration and stored at 4°C.

b) Preparing pre-inoculums and inoculums

Pre-inoculums were prepared with the same medium composition corresponding to the batch; the conserved microorganism was taken in solid sucrose medium, seeded in a volume of liquid medium, at 5-20% inoculum volume, cultured at 25-35 °C, with stirring at 100-400 rpm for 12-24 hours.

Composition of the medium used:

Component	**concentration (g/l)**
Salts:	
K_2HPO_4	10-20
$FeSO_4.\ 7H_2O$	0.03
$MgSO_4.\ 7H_2O$	0.02
$MnSO_4.\ H_2O$	0.002 – 0.1
$CaCl_2.\ 2\ H_2O$	0.0015 – 0.015
NaCl	0.01-0.1
Carbon source:	
Sucrose	15-30
Nitrogen source:	
Yeast extract	15-30

The microorganism was seeded at 5-10 % of the fermentation volume and grown up to an average optical density of around 0.7 absorbance units in 1:10 dilution, read at 600nm. A sterile culture medium was used as target.

A preinoculum and inoculum must be made during fermentation, depending on fermenter volume, in such a way that the necessary quantity of cells is obtained in final inoculum (10% culture medium deposited in the production fermenter) to avoid the latency phase in the reactor and trying to maintain the 1:10 volume ratio between the preinoculum and the inoculum or sufficient cell density to serve as inoculum, maintaining rigorous control over culture purity and vegetative state so that it can be used as either inoculum or preinoculum.

c) Preparing the culture medium and inoculation

Culture medium pH was adjusted to pH 7.0. The balloon flask containing the medium for preparing the preinoculum was sterilised at 121°C for 15 minutes.

d) Operating conditions

Active ingredient was produced by batch fermentation using the established medium. The operating conditions are listed in the following Table.

Fermenter operating conditions

Conditions	14 l
Medium volume (l)	10
Medium volume/fermenter volume ratio	0.8
Inoculum percentage	5-10
Inoculation optical density	0.5-1
Stirring (rpm)	100-400
Temperature (°C)	25-35
Aeration (vvm)	1-3
Initial medium pH	5-8
Fermentation time (hours)	6-12

2. Enzyme recovery:

a) Centrifuging

Extracellular enzyme was recovered by centrifuging at 5,000 rpm for 15 minutes to separate the biomass. The enzyme extract presented 2–6 U/ml glucosyltranspherase activity.

b) Ultrafiltration

Another way of recovering the fermentation supernatant is by using 0.45-2 micra pore size ultra filtration membranes.

Example 3

Biopolymer production and recovery

a) Enzymatic reaction. Reaction conditions were as follows:

Reactant medium:

50-200 Mm phosphate buffer pH	: 5 - 7
Substrate	: 8-20% sucrose
Enzyme quantity	: 10-30% v/v enzyme extract (200-500 U/l).
Reaction time	: 20-40 hours
Stirring	: 100-400 rpm

The enzyme was separated by centrifuging, placed in medium containing 8-20% sucrose, at pH 5-8 and at 25-35°C for 20-30 hours, obtaining 30-60 g/l polymer concentration corresponding to 40-60% yield regarding the substrate. In other reported processes 5-10 days for producing the polymer were needed. The reported microorganisms produced less polymer concentration (Table 1).

b) Purifying the biopolymer

The temperature was lowered to 4°C following the enzymatic reaction and it was possible to recover the biopolymer in two ways:

- **Precipitation with solvents**. 96% ethanol was added to the cold reaction mixture with stirring. The quantity of added ethanol corresponded to 1.0-3.0 volumes of ethanol/volume of the reaction mixture.
- The precipitated biopolymer was redissolved in half the volume of deionised and distilled water and is precipitated again with 1.0 to 3.0 volumes of ethanol/ reaction mixture volume.
- Precipitated biopolymer was redissolved in a third of the volume of water and dried by lyophilisation or dried by compressed air at 60-80°C until reaching 5-10% humidity.

Table 1

EPS production using different microorganisms

Organism	**Biopolymer (g/100 ml)**
Acetobacter pasteurianus	
ATCC 11142	0
B. polymyxa	
NRRL B-68	0
NRRL B-130	0
NRRL B-510	1.2

NRRL B-4317	1.4
Isolate (NRRL B-18475)	3.6
B. subtilis	
NRRL B-447	1.0
NRRL B-577	0
NRRL B-644	0
NRRL B-675	1.0
NRRL B-744a	1.5
NRRL B-2612	0
Enterobacter levanicum	
NRRL B-1678	0.7
Microbacterium laevaniformans	
ATCC 15953	1.2

- **Ultrafiltration.** The reaction mixture was submitted to ultrafiltration on a regenerated cellulose membrane having a pore size greater than 10,000 Daltons to eliminate residual glucose and fructose. The biopolymer was then dryied by aspersion process.

Biopolymer production by this microorganism depends on the substrate concentration, this being optimal at 8-24% where the biopolymer is produced having the greatest degree of purity with the greatest yield (Table 2).

Table 2

Effect of sucrose on biopolymer production by *Lactococcus lactis*

Sucrose (%)		**Biopolymer (g/l)**
Control	0	0% (sucrose free)
Sucrose	8	38.8
Sucrose	12	50.1
Sucrose	16	55.6

c) Drying

The obtained final product was a white powder which could be dried by lyophilisation or dry heat at a temperature not greater than 80°C.

Example 4

Biopolymer characterisation

1. Solubility

The product was a hydro-soluble biopolymer able to form hydrogel homogeneous dispersions up to 50% maximum concentration. 1.0 g of biopolymer was dissolved in 32 ml 5% chlorhydric acid, in 50 ml 10% sodium hydroxide or in 30 ml glacial acetic acid.

It was insoluble in ethanol, isopropanol, acetone, mineral and vegetal oil and polyethylen glycol.

The product was moderately soluble in 0.5% oxalic acid at at reflux temperature.

2. High Performance Liquid Chromatography (HPLC).

- A 1.5% biopolymer solution presented a 900-1,100 KDa molecular weight in permeation chromatography determined on a Shodex OHPak KB-803 column. Chromatography conditions were as follows:

Temperature : 55°C

Mobile phase : 0.1 M NaCl solution

Flow : 0.9 ml/min

- Polymer purity was greater than 95%, revealed by a thin peak in HPLC, in the following conditions:

Column: Shodex SC1011

Mobile phase: distilled deionised water

Flow: 0.6 ml/min.

Temperature: 70°C.

Equipment: Waters 510 with refraction index detector (Waters 2410).

The biopolymer presented a 7 to 7.5 minute retention time under these conditions.

The patterns used were analytic reagent grade glucose, fructose, sucrose and levan.

- The biopolymer was stable over a broad pH range revealed by HPLC following polymer contact with pH 2-9 buffers.

3. Viscosity

Viscosity was determined in a 10% solution at 30°C using a ViscoEasy viscosimeter L Series, Schott, Ref. 28.541.120, L2 stem at 50 rpm. The samples analysed presented viscosity ranging from 1,000-3,000 centipoises (cP). Pseudo-plastic behaviour was exhibited (cross-sectional thinning). Biopolymer solution viscosity lowered whenthe shear rate increases and increases on reducing temperature.

4. Dimensional characteristics

The biopolymer had a true density close to that of sucrose (1.5 mg/ml). It is a material showing high inter-particulate porosity (48%).

Average DVS particle size (diameter/volume/surface) was 224 micron.

5. Humidity adsorption

Water adsorption capacity ranged from 6.12 mg/g to 353.20 mg/g depending on relative humidity; this means that it was a slightly hygroscopic material. The biopolymer was able to soak up unlimited quantities of water due to its polymeric structure and hydrophilicity, being able to form variable consistency systems depending on the quantity of water incorporated, giving rise to the formation of aqueous dispersions characterised by their high viscosity.

6. Humidity

It presents losses of up to 10% when dried in a vacuum oven at 60°C.

7. Thermal characteristics

The biopolymer presents two glass transition points; the first between 20°C and 30°C and the second between 190°C and 220°C as determined by scanning differential calorimetry.

8. Microbiological quality

The biopolymer presents the following microbiological counts:

Microbiological charge	Range	Unit

Viable mesophile count	2000 - 4000	ufc / gr
Coliform count	Absence	nmp / gr
Faecal coliform count	<10	nmp / gr
Salmonella count	Absence	
mildew and yeast count	2000 - 5000	ufc / gr

9. Uses

a) The biopolymer could be used in the pharmaceutical industry as viscosant, thickener, stabiliser, dispersant, as a film former, as disintegrant, blood plasma substitute, lubrication agent and/or prebiotic agent.

b) The biopolymer could be used in the food industry as a thickener, viscosant, stabiliser, dispersant, as fibre and as fat , oils and ether- and ester-based carbohydrates substitute.

c) The biopolymer can be used in products obtained by extrusion, for forming films apt for producing flexible and biodegradable packages and for obtaining disposable biodegradable products, obtained by injection or moulding and in the production of flocculent agents for water treatment.